Published in Combination with SR 70-8 in
Laboratory Investigation
Vol. 25, No. 3, pp. 230-239, 1971

AFRRI SR69-26
DECEMBER 1969

**AFRRI**
**SCIENTIFIC**
**REPORT**



# RADIATION-INDUCED ULTRASTRUCTURAL CHANGES IN LYSOSOMES

## I. Cytochemical Analysis

AFRRI SR69-26

**ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE**
**Defense Atomic Support Agency**
**Bethesda, Maryland**

This report has been approved for open publication by the Department of Defense

All aspects of investigative programs involving the use of laboratory animals sponsored by DOD components are conducted according to the principles enunciated in the "Guide for Laboratory Animal Facilities and Care", prepared by the National Academy of Sciences - National Research Council.

AFRRI SR69-26
December 1969

# RADIATION-INDUCED ULTRASTRUCTURAL CHANGES IN LYSOSOMES

## I. CYTOCHEMICAL ANALYSIS

A. A. RENÉ
J. L. PARKER
J. H. DARDEN

S. J. BAUM
Chairman
Experimental Pathology Department

HUGH B. MITCHELL
Colonel, USAF, MC
Director

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Atomic Support Agency
Bethesda, Maryland

---

## ACKNOWLEDGMENT

We wish to express our thanks to S. J. Baum and R. M. Nardone for their helpful suggestions and advice.

TABLE OF CONTENTS

LIST OF FIGURES

## FOREWORD
(Nontechnical summary)

All cells, with the exception of bacteria and mature red cells are composed of a nucleus and cytoplasm. These two main cell parts contain specialized organelles and structures which function to perpetuate the life of the cell. One of these organelles, the lysosome, is a small cytoplasmic particle containing a variety of enzymes which are capable of breaking down fats, carbohydrates and proteins. Thus the particle has a digestive function in the cell. These lysosomal enzymes, which are bound by the single membrane of the lysosome, can cause damage to the cell if this membrane becomes so altered as to allow their uncontrollable escape into the cytoplasm. The membrane can be made more permeable by treatment with a variety of substances and conditions, which includes radiation. Indeed, enzymes released from radiation-damaged lysosomes could in turn contribute to cellular injury or death.
In the present study a correlation was obtained of the changes which occur in lysosomes after irradiation with the damage which takes place in the cells. The use of an enzyme marker suggested that radiation causes an initial "build-up" of the enzyme within the lysosome 2 hours after irradiation. The possibility exists that this was followed by an enzyme release which could be responsible for cell damage.

## ABSTRACT

Ultrastructural and biochemical changes in lysosomes of rat liver following exposure to ionizing radiation were studied. A marker for acid phosphatase was used to visually correlate the progressive changes in lysosomes with the cellular necrobiotic process postirradiation. The earliest observable change in the lysosomes and/or lysosomal enzymes corresponding with the sequence of fine structural alterations following irradiation suggests that radiation labilizes the lysosomal membrane resulting in a release of enzymes responsible for cell damage. The concentration of the lead phosphate reaction product indicated that the initial action on the lysosome is evidently a "build-up" of hydrolytic enzymes within 2 hours after irradiation followed by a gradual release of the marked enzyme 2-24 hours postirradiation as noted by decreased enzyme concentration within the lysosomes. The release of the enzyme appeared to be directly related to an increasing cellular necrobiosis following irradiation.

# I. INTRODUCTION

Lysosomes have been identified as distinct intracellular structures bound by a single membrane and containing enzymes. Upon release from the lysosome, these enzymes are capable of causing repairable or irreparable cellular damage.[11] The increase in activity of a number of specific lysosomal enzymes in isolated cells or tissues of irradiated animals has been widely reported.[4,6,7,15,16,22,24] Isolated lysosomes irradiated in vitro showed no distinguishable effects, even at very high doses (1-50 krads).[6] Thus, it has been suggested that the release of the lysosomal enzymes postirradiation was mediated by conditions that require the integrity of the cells.[22] It seemed logical then that clarification on whether or not lysosomes caused damage to irradiated cells would have to be derived from in vivo studies.

An in vivo system to study radiation-induced lysosomal changes has been used by a number of investigators.[2,10,19] More specific studies have been made through the use of a marker for acid phosphatase which made it possible to visually associate the lysosomes with cellular radiation lesions.[3,9,14,18] This test seems to be sufficiently sensitive to detect the differences which prevail between the irradiated and control lysosomal concentration of acid phosphatase.

The objective of the present study is to correlate the progressive changes which occur in lysosomes after irradiation with the postirradiation necrobiotic process which takes place in cells. More specifically, a comparison of the earliest observable change in the lysosomes and/or lysosomal enzyme activity with the sequence of fine structural alteration following irradiation in cells was undertaken.

## II. MATERIALS AND METHODS

Eighty Sprague-Dawley rats of the Charles River strain, weighing approximately 200 grams were used. Thirty-two animals were irradiated and the remaining forty-eight animals were used in equal numbers as starved and fed unirradiated controls. The experimental population of rats was exposed to 2 krads of whole-body x radiation from a Maxitron with the following physical parameters: 250 kVp, 30 mA, filtered by 1.2 mm Be and 0.95 mm Cu; HVL - 1.9 mm Cu. The distance of the x-ray tube from the animal midline was 60 cm. For the radiation exposure, all rats of an exposure group were placed in Lucite boxes and arranged in the radiation field so that the tissue dose rate to the midline of the exposure volume was similar for all rats (maximum deviation ± 4 percent).

The irradiated and control animals were anesthetized with 0.25 ml of Sodium Nembutal (50 mg/ml) and the liver perfused with 3.0 percent glutaraldehyde buffered with .067 M cacodylate pH 7.4. The liver specimen was rapidly removed, cut into strips (2 x 5 x 10 mm) and washed overnight at 4°C in 0.1 M cacodylate containing 7.5 percent sucrose. The sections were subsequently mounted in agar and cut into 50 μm sections with a Sorvall tissue sectioner. The sections were incubated for 30 minutes at 37°C in a substrate medium (Gomori method, Barka and Anderson[1]) containing β-glycerophosphate (Grade III, Sigma Chemical Co., St. Louis, Missouri) pH 5.0. The liver specimens, used as controls for those incubated in the substrate medium, were incubated in the medium minus β-glycerophosphate.

The sections were washed in Tris buffer containing 7.5 percent sucrose, pH 5.0 following the incubation procedure. The sections were then postfixed in 1 percent

osmium tetroxide,[12] dehydrated in graded ethanol solutions and embedded in Maraglas.[23] The blocks were cut with a Porter-Blum MT2 ultramicrotome and sections mounted on uncoated grids. After staining with uranyl acetate[20] and lead citrate[17] the sections were examined in a Siemens electron microscope.

## III. RESULTS

An electron micrograph of a normal hepatic lysosome is illustrated in Figure 1. In comparison, lysosomes incubated in acid phosphatase substrate medium show lysosomal aggregates in the vicinity of the bile canaliculus with electron dense areas of the reaction product, lead phosphate (Figure 2). The lysosomes (L) are intact with single membranes, showing some variation in the internal structure and number. The areas of lead salt deposits, usually spherical in nature, are normally uniformly dense and eccentrically located in the lysosomes. Exceptions to this are illustrated

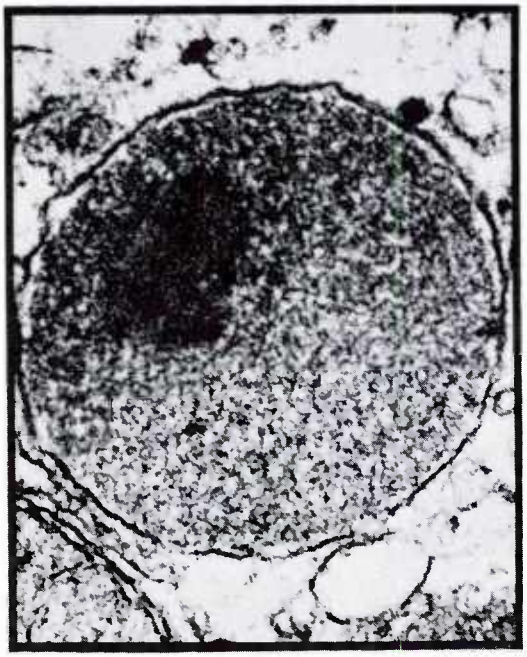

Figure 1. Lysosome of a normal rat liver cell. X 100,000

in Figure 2b, in which a dark area can be seen with a very light matrix and in Figure 2c (arrow) in which the reaction product can be seen as a fibrous material.

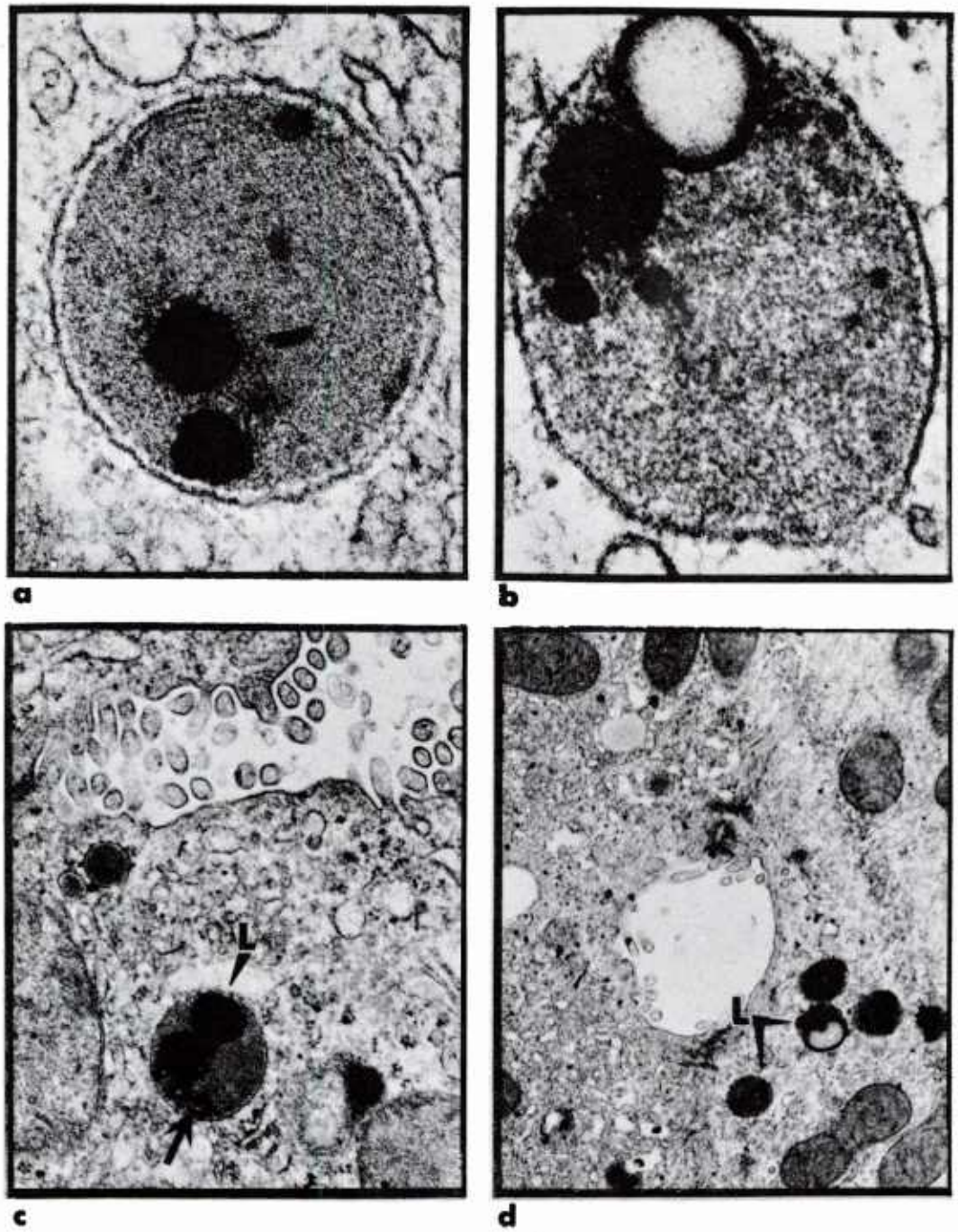


Figure 2. Normal lysosomes of rat liver cells incubated in β-glycerophosphate. The electron dense areas indicate the presence of acid phosphatase. (a, b, c, and d present different sections of the same specimen.) (a) X 110,000; (b) X 115,000; (c) X 32,500; (d) X 16,250

There was no difference between the starved and fed unirradiated controls and they were used interchangeably. The hepatocytes of animals sacrificed immediately after irradiation (1-2 hours) exhibited little change in morphology when compared to the normal cells (Figure 3). The reaction product of the lysosomes in some instances appeared to be more dense and covered a larger area. Some of these areas almost completely covered the whole lysosome (Figure 3a). In other cells, the reaction product was more fibrous or appeared in greater numbers of individual inclusion (Figure 3c and d). The fine structure of the cell appeared to be normal in all other aspects. The hepatocytes of the animals sacrificed 24 hours after irradiation were morphologically similar to the hepatocytes of the animals sacrificed earlier. The lysosomes of these two groups of animals were very much alike, although there appeared to be a decrease in the concentration of the reaction product (Figure 4). The fine structure of the hepatocytes gave initial evidence of cellular necrosis and the appearance of glycogen (G).

The electron micrographs of the specimen collected 48 hours after irradiation began to show marked changes in fine structure (Figure 5). The most apparent changes were: (1) the appearance of multivesicular bodies; (2) the formation of myelin-like figures in and around prominent glycogen areas (rosettes); (3) the reduction and quite often the absence of electron dense areas of reaction product in most lysosomes. This was accompanied with evidence of membrane degradation and a clearing of the lysosomes.

The most striking change in the fine structure of the hepatocytes of the animals 72 hours after irradiation is illustrated in Figure 6. The lysosomes exhibited

marked evidence of deterioration with membrane budding, breaking and matrix clearing. A few lysosomes still had dense peripheral areas. In some areas the lysosomes could be seen as aggregates, especially in the necrobiotic areas. The material which composed its matrix gave the morphological appearance of glycogen (Figure 6b). Some of the lysosomes which gave no evidence of the presence of the reaction product assumed the role of autophagic vacuoles (Figure 6a, b, c). The cytoplasm showed evidence of a reduced amount of glycogen and extreme deterioration of fine structure. Areas of the rough endoplasmic reticulum were without granules in some instances. The mitochondria did not show evidence of damage. The mitochondrial granules and membranes appeared normal except those externally associated with myelin-like figures (arrow) where degradation of the mitochondria occurred (Figure 6d).

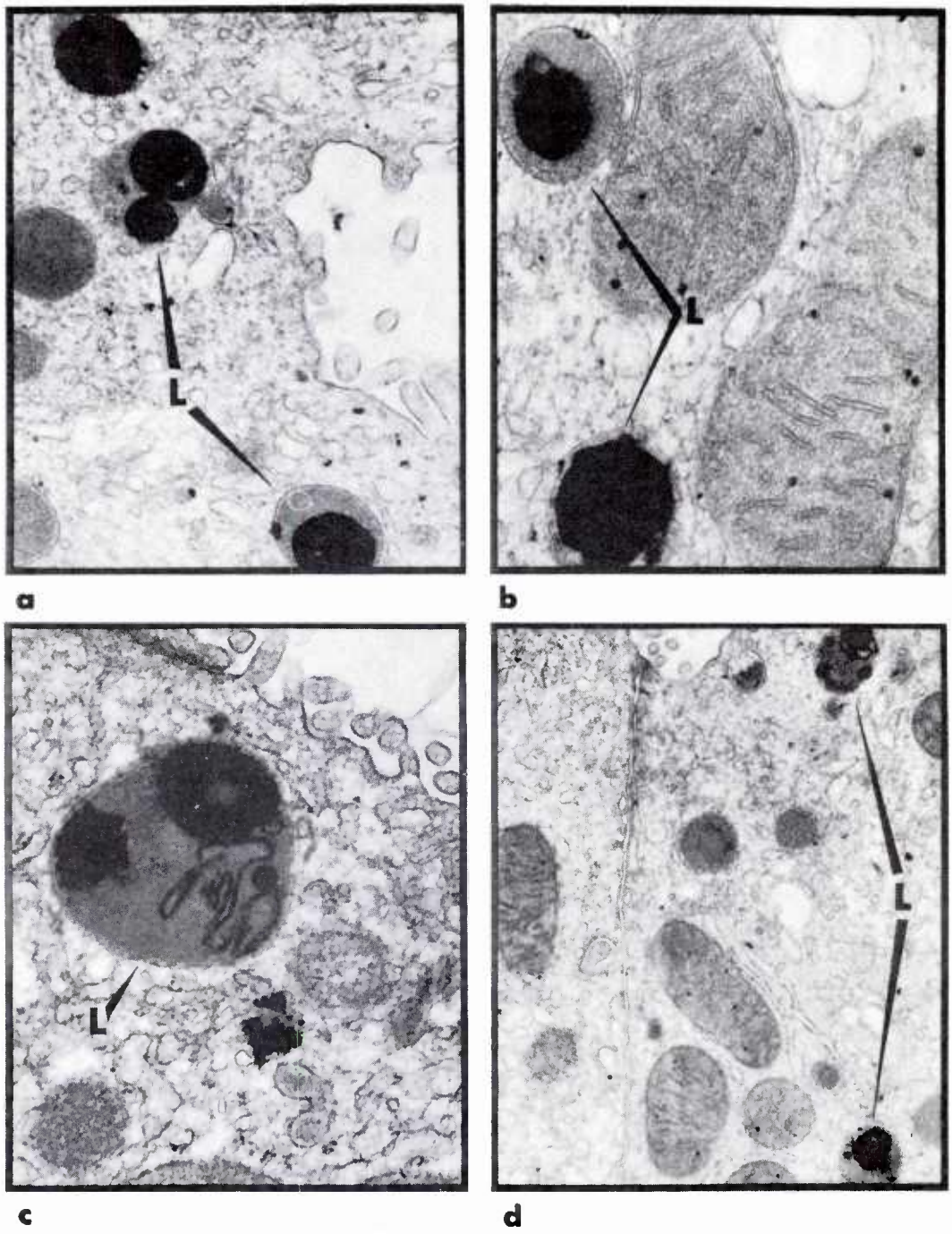


Figure 3. Lysosomes of liver cells of rats sacrificed 2 hours after exposure to 2 krads of x rays. The liver specimen was incubated in $\beta$-glycerophosphate to indicate the presence of acid phosphatase. (a, b, c, and d present different sections of the same specimen.) (a) X 31,200; (b) X 49,000; (c) X 40,000; (d) X 20,000

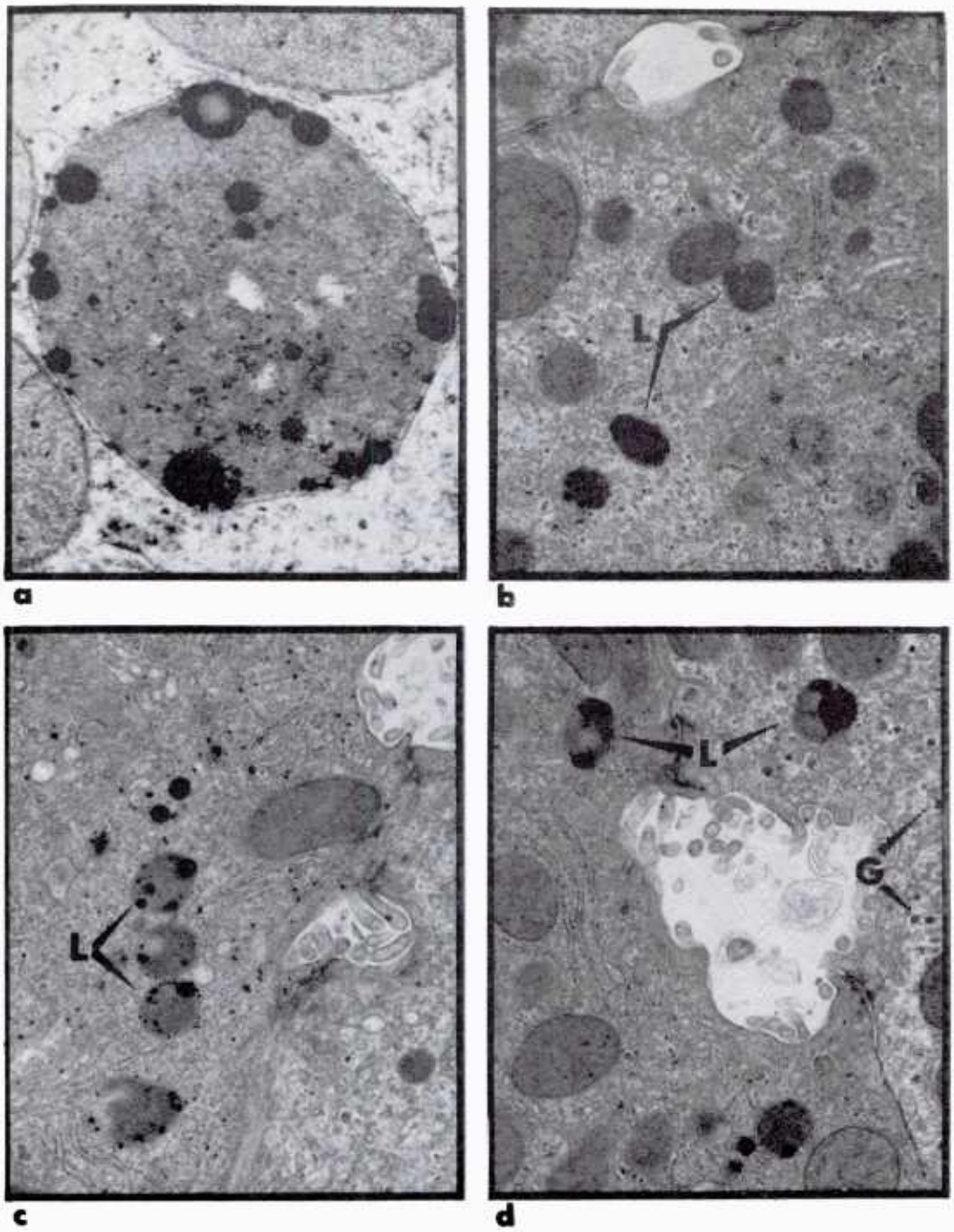


Figure 4. Lysosomes of liver cells of rats sacrificed 24 hours after exposure to 2 krads of x rays. The liver specimen was incubated in β-glycerophosphate to indicate the presence of acid phosphatase. (a, b, c, and d present different sections of the same specimen.) G = glycogen. (a) X 42,500; (b) X 21,250; (c) X 21,250; (d) X 21,250

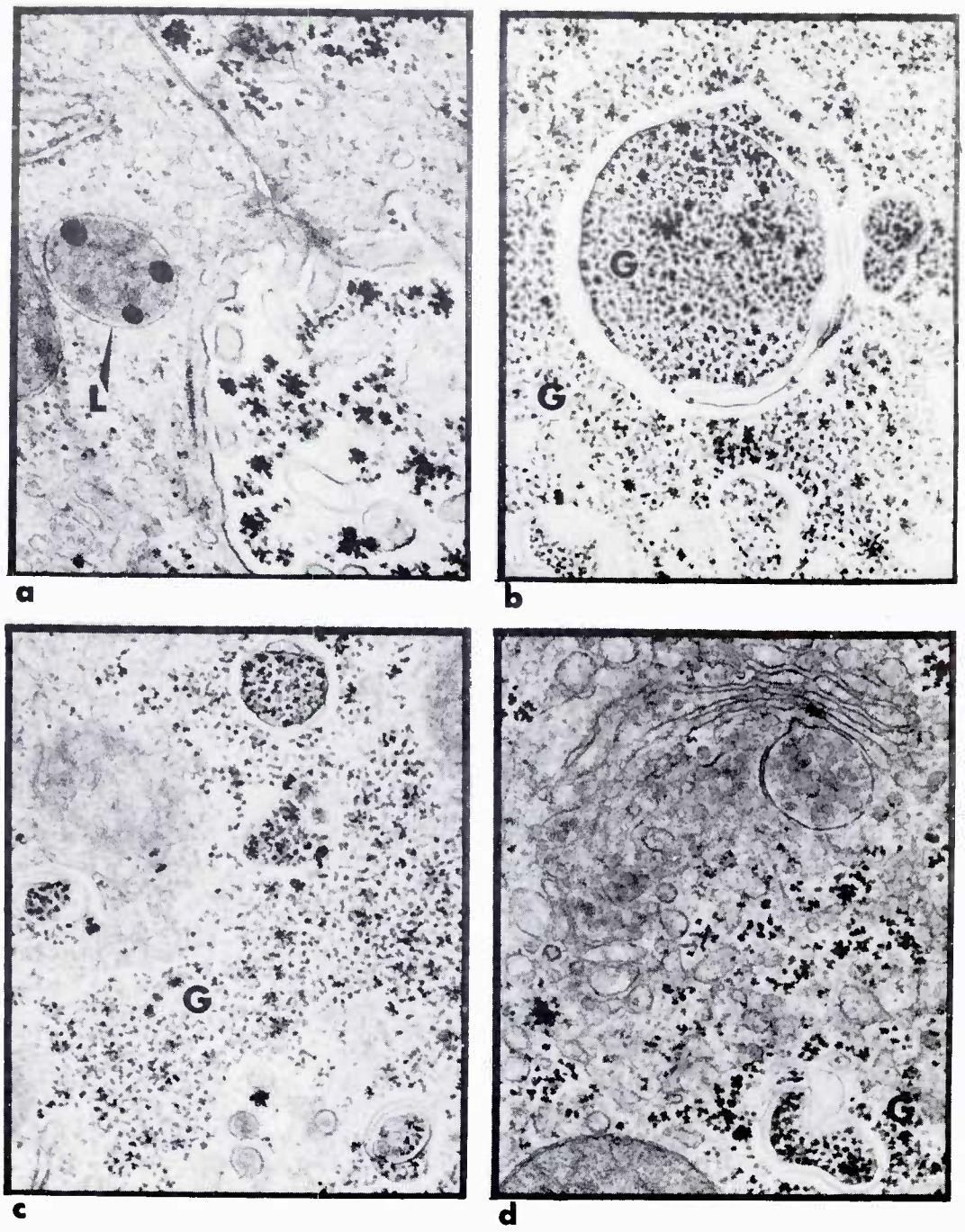


Figure 5. Specimen of liver cells of rats sacrificed 48 hours after exposure to 2 krads of x rays. The liver specimen was incubated in $\beta$-glycerophosphate to indicate the presence of acid phosphatase. (a, b, c, and d present different sections of the same specimen.) G = glycogen. (a) X 42,500; (b) X 37,500; (c) X 37,500; (d) X 37,500

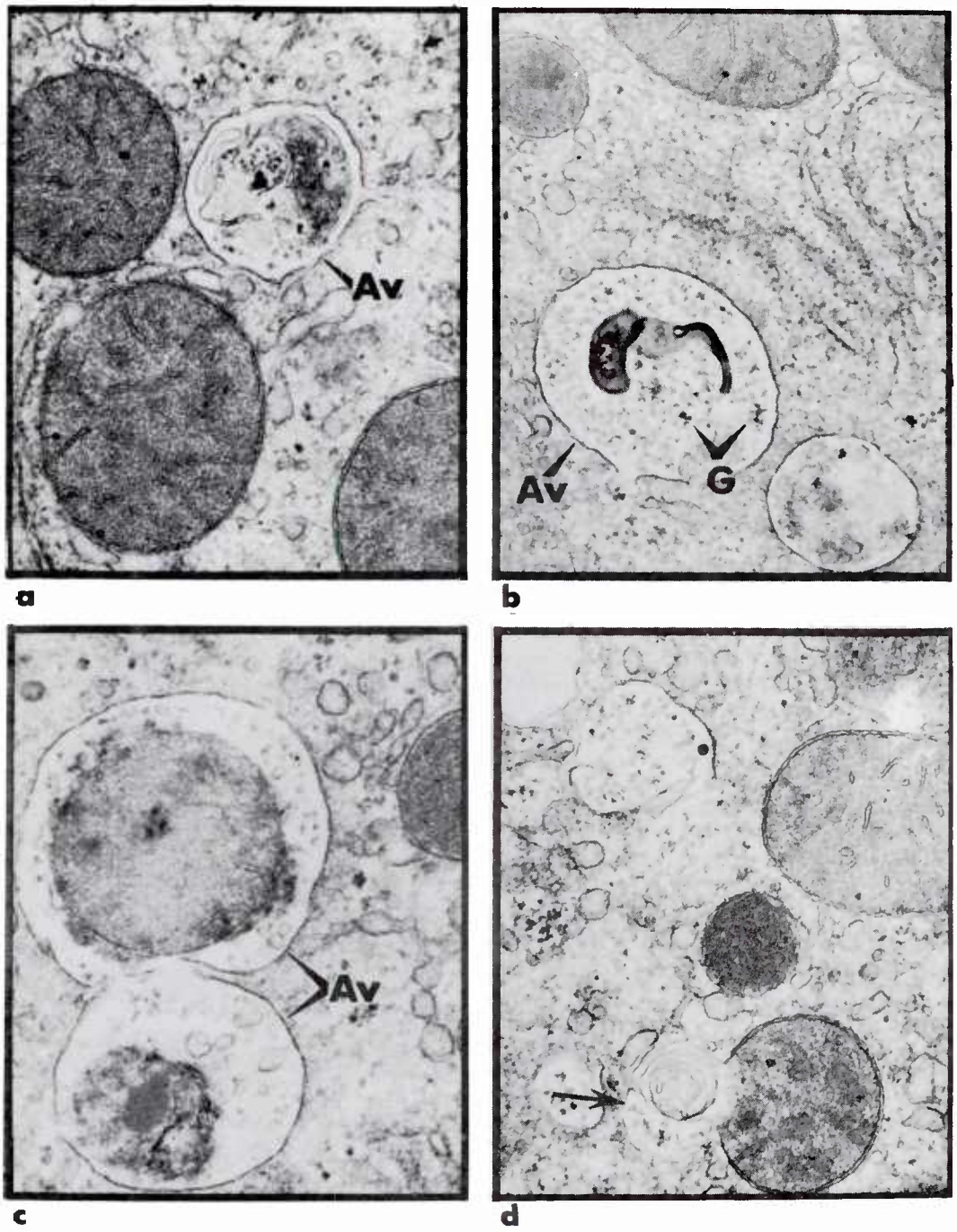


Figure 6. Specimen of liver cells of rats sacrificed 72 hours after exposure to 2 krads of x rays. The liver specimen was incubated in $\beta$-glycerophosphate to indicate the presence of acid phosphatase. (a, b, c, and d present different sections of the same specimen.) Av = autophagic vacuoles. (a) X 37,500; (b) X 37,500; (c) X 37,500; (d) X 37,500

## IV. DISCUSSION

It has been suggested that if lysosomes were the cause of radiation injury in cells then we should find some changes in the lysosomes occurring before the onset of a visible cellular injury.[21] The results of the present cytochemical study indicated that lysosomes of hepatocytes, under the experimental conditions described, are susceptible to radiation effects which are observable within 2 hours after irradiation (Figure 7). This, however, was manifested as an increase in hydrolytic enzyme activity (reaction product, lead phosphate) and not observed as an alteration in lysosomal fine structure nor was it accompanied by any visible changes in the cytoplasmic ultrastructure. This early increase in enzyme activity has been reported by others. In some cases this was observed as early as 30 minutes postirradiation[9] and in other instances not until 16 hours postirradiation.[19] The significance of this "build-up" phenomenon is not yet known. It has been reported, however, that frog neuronal lysosomes showed variable deterioration (swelling and clearing) after exposure to 1000-2000 R x rays, which is probably due to an increased permeability of the lysosomal membrane, causing entry of fluids.[13] Pronounced escape of lysosomal enzymes into the cytoplasm was questionable.

Evidence for enzyme release was reported by Brandes et al.,[3] who observed the presence of the reaction product in the cytoplasm and in intercellular spaces 3 days after exposure to radiation. However, enzymes could be released and might not be detected in the cytoplasm. As a matter of fact, the presence of a concentrated form of reaction product outside the lysosomes within the matrix of the cytoplasm at any time after irradiation was not expected in the present study. The enzyme

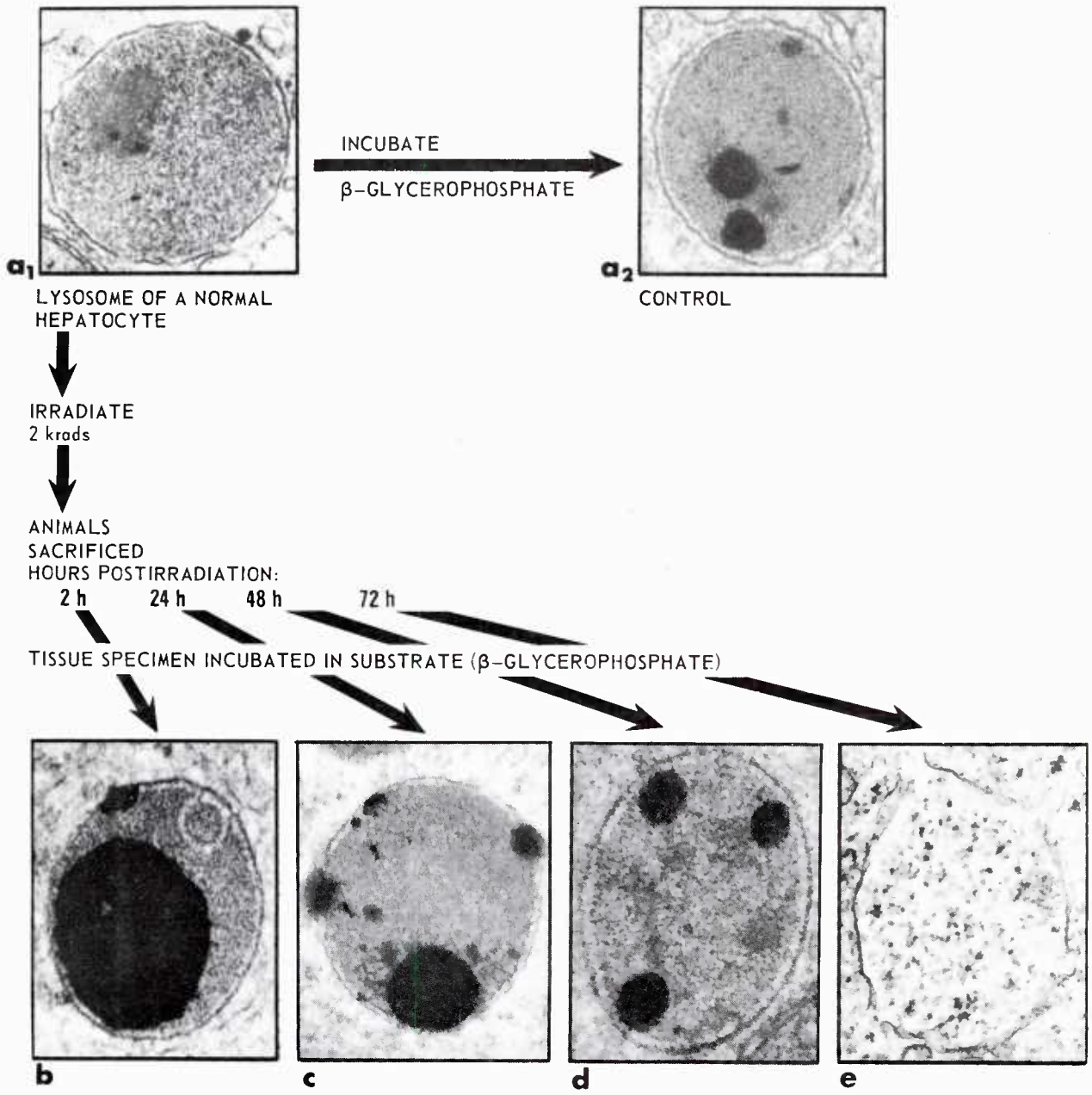


Figure 7. Schematic representation of lysosomal changes following x irradiation. $a_1$ is a lysosome of a normal hepatocyte. $a_2$ is a lysosome of a normal hepatocyte after incubation in the substrate medium $\beta$-glycerophosphate. b, c, d, and e represent lysosomes at various periods after irradiation, 2, 24, 48 and 72 hours, respectively. At b there is a "build-up" of reaction product which decreases at c and d. There is a loss of membrane competence 72 hours after irradiation (e) allowing the free movement of material across the membrane, resulting in a lysosomal clearing phenomenon.

upon leaving the relatively small lysosomal particle and entering the cytoplasmic matrix is immensely diluted and would not necessarily be visible as dense lead salt deposits.

Alterations in the fine structure of the cell within 2 hours after irradiation were not apparent. Ultrastructural alterations have been identified within parenchymal cells of liver tissue as early as 2 minutes after exposure[8] where the doses used were up to 16 krads. That study could not be correlated with the present study because of the significant difference in the doses used.

The number and size of lysosomes do not show a notable change 24 hours after irradiation, yet, there was a decrease of the reaction product (Figure 7). This condition could be the result of a release of lysosomal enzymes in view of the fact that we also began to observe signs of cellular atrophy which were especially prominent in specimens collected 48 hours after exposure. The appearance of glycogen cannot be ascribed to an injury phenomenon. The rosettes are more evident in the liver of animals that are briefly fasted or subjected to other experimental treatments which result in the depletion of carbohydrate stores.[5] The abundance of glycogen, therefore, could be ascribed to inanition in the animals with severe radiation sickness. The appearance of annuli surrounding areas of dense glycogen rosettes can only be explained in terms of their relationship with an overall deterioration of the cell. Annuli apparently formed from the endoplasmic reticulum were reported in liver cells as early as 2 minutes after exposure,[8] but were not observed in our specimen until 48 hours after exposure. The clearing of lysosomes and breakdown of their membranes, apparent by the 3rd day after exposure, was probably the

most convincing evidence of a possible release of lysosomal enzymes capable of breaking down all the major constituents of the cell.

The formation of radicals is a well-known effect of radiation on an aqueous environment such as the cell. It has been suggested that irradiation causes the formation of lipid peroxides in lysosomes which leads to rupture of the lysosomal membrane and allows the release of the hydrolytic enzymes.[24] It has also been suggested that the formation of free radicals is mediated through a combination of lipid and oxygen which act as free radical initiators to form unstable peroxides. Lysosomes are particularly labile to peroxides.[21] The lysosomal membrane was found extremely labile when isolated and subjected to hydrogen peroxide.[4] In addition to the proposed effect of free radicals, Rahman[15] suggested that the action on the membrane was mediated through a hormonal action which occurs in vivo. A subsequent test of this theory with a thyrotropic hormone was positive.[16] Perhaps this is what Sottocasa[22] had in mind when he suggested that the release of the enzymes required the structural integrity of the cell.

The selective entry of fluids could explain the increase in size of lysosomes after irradiation reported by Brandes et al.[3] and Pipan.[14] The increase in the number of lysosomes could not be explained, except perhaps radiation has a stimulatory effect on the sites of lysosomal formation. The many small lysosomes could be newly formed ones.

In the present report, it is suggested that free radicals are formed in the hepatocytes immediately following irradiation which labilizes the lysosomal membrane and makes it more permeable. To what extent hormones are involved is not understood.

Initially there is a build-up of hydrolytic enzymes in the lysosomes. This phenomenon was reported by Pick[13] and is viewed as an activation of hydrolytic enzymes followed by a gradual selective release which becomes apparent after 2 hours postirradiation. This eventually leads to cellular necrobiosis.

## REFERENCES


1. Barka, T. and Anderson, P. J. Histochemistry: Theory, Practice and Bibliography. New York, Evanston, and London, Harper and Row Publishers, Inc., 1963.

2. Bouma, J. M. W. and Gruber, M. The distribution of cathepsins B and C in rat tissues. Biochim. Biophys. Acta 89:545-547, 1964.

3. Brandes, D., Sloan, K. W., Anton, E. and Bloedorn, F. The effect of X-irradiation on the lysosomes of mouse mammary gland carcinomas. Cancer Res. 27:731-746, 1967.

4. Desai, I. D., Sawant, P. L. and Tappel, A. L. Peroxidative and radiation damage to isolated lysosomes. Biochim. Biophys. Acta 86:277-285, 1964.

5. Fawcett, D. W. An Atlas of Fine Structure: The Cell, Its Organelles and Inclusions. Philadelphia, Pennsylvania, W. B. Saunders Company, 1966.

6. Harris, J. W. Response of isolated leukocyte lysosomes to gamma irradiation. Radiation Res. 28:766-778, 1966.

7. Harris, J. W. Studies of irradiated lysosomes with particular reference to the action of calcium. Exptl. Cell Res. 45:487-489, 1967.

8. Hendee, W. R. and Alders, M. A. Ultrastructural development of radiation injury in hepatic parenchymal cells of $\gamma$-irradiated mice. Lab. Investigation 18(2):151-158, 1968.

9. Hugon, J. and Borgers, M. Étude morphologique et cytochimique des cytolysomes de la crypte duodénale de souris irradiée par rayons X. Journal de Microscopie 4:643-656, 1965.

10. Noaman, Mohamed Ahmed-Attia. Effect of radiation and radioprotectors on lysosomal and other enzymes. Thesis, Athens, Georgia, University of Georgia, 1966. Nucl. Sci. Abstr. 22:1090 (Abstract 10738), 1968.

11. Novikoff, A. B., Beaufay, H. and de Duve, C. Electron microscopy of lysosome-rich fractions from rat liver. J. Biophys. Biochem. Cytology 2(4), Supplement:179-184, 1956.

12. Palade, G. E. A study of fixation for electron microscopy. J. Exptl. Med. 95:285-298, 1952.

13. Pick, J. The fine structure of sympathetic neurons in x-irradiated frogs. J. Cell Biol. 26:335-351, 1965.

14. Pipan, N. Elektronenmikroskopische Untersuchungen über die Strahlenwirkung auf die Leberzellen von Mäusefeten. Strahlentherapie 133:268-279, 1967.

15. Rahman, Y. E. Effect of X-irradiation on the fragility of rat spleen lysosomes. Radiation Res. 20:741-750, 1963.

16. Rahman, Y. E. A note on acid phosphatase release from spleen, liver and thymus of rats. Biochim. Biophys. Acta 90:440-442, 1964.

17. Reynolds, E. S. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17:208-221, 1963.

18. Roizin, L., Rugh, R. and Kaufman, M. A. Irradiation effects upon the fetal central nervous system of Macacus rhesus monkeys -- effects on lysosomes. Acta Radiol. (Therapy) 5:161-176, 1966.

19. Roth, J. S., Bukovsky, J. and Eichel, H. J. The effect of whole-body X-irradiation on the activity of some acid hydrolases in homogenates and subcellular fractions of rat spleen. Radiation Res. 16:27-36, 1962.

20. Sjöstrand, F. S. A new ultrastructural element of membranes in mitochondria and of some cytoplasmic membranes. J. Ultrastructure Res. 9:340-361, 1963.

21. Slater, T. F. Lysosomal changes in experimentally induced liver injury. Proc. Roy. Soc. Med. 59:877-880, 1966.

22. Sottocasa, G. L., Glas, G. and de Bernard, B. The effect of X-irradiation on the activities of some lysosomal hydrolases of heart tissue. Radiation Res. 24:32-42, 1965.

23. Spurlock, B. O., Kattine, V. C. and Freeman, J. A. Technical modifications in Maraglas embedding. J. Cell Biol. 17:203-207, 1963.

24. Wills, E. D. and Wilkinson, A. E. Release of enzymes from lysosomes by irradiation and the relation of lipid peroxide formation to enzyme release. Biochem. J. 99:657-666, 1966.

DISTRIBUTION LIST

AIR FORCE

The Surgeon General, U. S. Department of the Air Force. Washington, D. C. 20333 (1)
Executive Officer, Director of Professional Services, Office of the Surgeon General, Hq. USAF (AFMSPA) T-8, Washington, D. C. 20333 (1)
Headquarters, U. S. Air Force (AFMSPAB), Washington, D. C. 20333 (1)
USAFSAM (SMBR), ATTN: Chief, Radiobiology Branch, Brooks AFB, Texas 78235 (1)
Air Force Weapons Laboratory, ATTN: WLIL (1), ATTN: WLRB-2 (1), Kirtland AFB, New Mexico 87117 (2)
Chief, Nuclear Medicine Department, P. O. Box 5088, USAF Hospital, Wright-Patterson AFB, Ohio 45433 (1)
Office of the Command Surgeon (ADCSG), Hq. ADC, USAF, Ent AFB, Colorado 80912 (1)
Commander, 6571st Aeromedical Research Laboratory, Holloman AFB, New Mexico 88330 (2)

ARMY

The Surgeon General, U. S. Department of the Army, Washington, D. C. 20315 (1)
Surgeon Ceneral, ATTN: MEDDH-N, U. S. Department of the Army, Washington, D. C. 20315 (1)
USACDC CSSG, Doctrine Division, Fort Lee, Virginia 23801 (1)
CG, USCONARC, ATTN: ATUTR-TNG (NBC), Fort Monroe, Virginia 23351 (1)
Commanding Officer, U. S. Army Medical Research Laboratory, Fort Knox, Kentucky 40121 (1)
Commanding Officer, USA Nuclear Medical Research Detachment, Europe, APO New York, New York 09180 (2)
Army Research Office, ATTN: Chief, Scientific Analysis Branch, Life Sciences Division, 3045 Columbia Pike, Arlington, Virginia 22204 (1)
Division of Nuclear Medicine, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D. C. 20012 (5)
Commanding Officer, U. S. Army Environmental Hygiene Agency, ATTN: USAEHA-RP, Edgewood Arsenal, Maryland 21010 (1)
Commandant, U. S. Army Medical Field Service School, ATTN: MEDEW - ZNW, Fort Sam Houston. Texas 78234 (1)

NAVY

Chief, Bureau of Medicine and Surgery, U. S. Navy Department, Washington, D. C. 20390 (1)
Chief, Bureau of Medicine and Surgery, ATTN: Code 71, U. S. Navy Department, Washington, D. C. 20390 (1)
Director, Biological Sciences Division, Office of Naval Research, Washington, D. C. 20360 (1)
Commanding Officer, Naval Aerospace Medical Institute, NAMC, ATTN: Research Director, Pensacola, Fla. 32512 (3)
Head, Animal Behavioral Sciences Branch, Naval Aerospace Medical Institute, Naval Aerospace Medical Center, Pensacola, Florida 32512, ATTN: Dr. John S. Thach, Jr. (1)
Commanding Officer, U. S. Naval Hospital, ATTN: Director, REEL, NNMC, Bethesda, Maryland 20014 (1)
Commanding Officer, Nuclear Weapons Training Center, Atlantic, Nuclear Warfare Department, Norfolk, Virginia 23511 (1)

D. O. D.

Director, Defense Atomic Support Agency, Washington, D. C. 20305 (1)
Director, Defense Atomic Support Agency, ATTN: DDST, Washington, D. C. 20305 (1)
Director, Defense Atomic Support Agency, ATTN: Chief, Medical Directorate, Washington, D. C. 20305 (4)
Director, Defense Atomic Support Agency, ATTN: Technical Library (APTL), Washington, D. C. 20305 (2)
Commander, Field Command, Defense Atomic Support Agency, ATTN: FC Technical Library, Sandia Base, Albuquerque, New Mexico 87115 (1)
Director, Armed Forces Institute of Pathology, Washington, D. C. 20305 (1)
Administrator, Defense Documentation Center, Cameron Station, Bldg. 5, Alexandria, Virginia 22314 (20)

OTHER GOVERNMENT

U. S. Atomic Energy Commission, Headquarters Library, Reports Section, Mail Station G-17, Washington, D. C. 20545 (1)
U. S. Atomic Energy Commission, Division of Biology and Medicine, Washington, D. C. 20545 (1)

OTHER GOVERNMENT (continued)

U. S. Atomic Energy Commission, Bethesda Technical Library, 7920 Norfolk Avenue, Bethesda, Maryland 20014 (1)
National Aeronautics and Space Administration, ATTN: Lt. Col. Charles M. Barnes, USAF, DB-3, MSC, Houston, Texas 77058 (1)
National Bureau of Standards, ATTN: Chief, Radiation Physics Division, Washington, D. C. 20234 (1)
U. S. Public Health Service, Deputy Chief, Division of Radiological Health, Washington, D. C. 20201 (1)
U. S. Public Health Service, Radiological Health Laboratory, ATTN: Library, 1901 Chapman Avenue, Rockville, Maryland 20852 (1)
U. S. Public Health Service, Northeastern Radiological Health Laboratory, 109 Holton Street, Winchester, Massachusetts 01890 (1)
U. S. Public Health Service, Southwestern Radiological Health Laboratory, P. O. Box 684, Las Vegas, Nevada 89101 (1)
U. S. Public Health Service, National Center for Radiological Health, Information Office, Room 3, Twinbrook Laboratory, RBE Program, 1901 Chapman Avenue, Rockville, Maryland 20852 (1)

OTHER

Argonne National Laboratory, Library Services Department, Report Section Bldg. 203, RM-CE-125, 9700 South Cass Avenue, Argonne, Illinois 60440 (1)
Dr. Donald G. Baker, Radiobiology Department, Zellerbach Saroni Tumor Institute, 1600 Divisadero Street, San Francisco, California 94115 (1)
Brookhaven National Laboratory, Information Division, ATTN: Research Library, Upton, Long Island, New York 11973 (2)
Dr. J. S. Burkle, Director of Nuclear Medicine, York Hospital, York, Pennsylvania 17403 (1)
Director, Radiobiology Laboratory, University of California, Davis, California 95616 (1)
University of California, Lawrence Radiation Laboratory, Library, Bldg. 50, Room 134, Berkeley, Calif. 94720 (1)
University of California, Lawrence Radiation Laboratory, Technical Information Division Library L-3, P. O. Box 808, Livermore, California 94551 (2)
University of California, Laboratory of Nuclear Medicine and Radiation Biology, Library, 900 Veteran Avenue, Los Angeles, California 90024 (1)
Director, Collaborative Radiological Health Laboratory, Colorado State University, Fort Collins, Colorado 80521 (1)
Dr. L. W. Davis, Radiology Department, University of Pennsylvania, 3400 Spruce Street, Philadelphia, Pa. 19104 (1)
Professor Merril Eisenbud, New York University, Tuxedo, New York 10987 (1)
Dr. T. C. Evans, Radiation Research Laboratory, College of Medicine, University of Iowa, Iowa City, Iowa 52240 (1)
Dr. Arnold Feldman, Institute of Radiology, School of Medicine, Washington University, 510 South Kingshighway, St. Louis, Missouri 63110 (1)
Mr. Orin Gelderloos, Department of Biological Sciences, Northwestern University, Evanston, Illinois 60201 (1)
General Dynamics/Fort Worth, ATTN: Librarian, P. O. Box 748, Fort Worth, Texas 76101 (1)
Gulf General Atomic Incorporated, ATTN: Library, P. O. Box 608, San Diego, California 92112 (1)
Hazleton Nuclear Science Corporation, ATTN: Library, 4062 Fabian Way, Palo Alto, California 94303 (1)
IIT Research Institute, ATTN: Document Library, 10 West 35th Street, Chicago, Illinois 60616 (1)
Dr. R. F. Kallman, Department of Radiology, Stanford University, Palo Alto, California 94305 (1)
Dr. L. S. Kelly, Donner Laboratory, University of California at Berkeley, Berkeley, California 94720 (1)
Los Alamos Scientific Laboratory, ATTN: Report Librarian, P. O. Box 1663, Los Alamos, New Mexico 87544 (1)
Director, Nuclear Science Center, Louisiana State University, Baton Rouge, Louisiana 70803 (2)
Lovelace Foundation for Medical Education & Research, Document Library, 5200 Gibson Boulevard, S. E. Albuquerque, New Mexico 87108 (1)
Dr. Ross A. McFarland, Guggenheim Professor of Aerospace Health & Safety, Harvard School of Public Health, 665 Huntington Avenue, Boston, Massachusetts 02115 (1)
Dr. J. I. Marcum, Rand Corporation, 1700 Main Street, Santa Monica, California 90401 (1)
Massachusetts Institute of Technology, M.I.T. Libraries, Technical Reports, Room 14 E-210, Cambridge, Massachusetts 02139 (1)
Dr. Charles W. Mays, Physics Group Leader, Radiobiology Division, University of Utah, Salt Lake City, Utah 84112 (1)
Dr. B. D. Newsom, Colony Oaks, Apt. 32, 18100 Nassau Bay Drive, Nassau Bay, Texas 77058 (1)
Ohio State University, Nuclear Reactor Laboratory, 1298 Kinnear Road, Columbus, Ohio 43212 (1)
Dr. Harvey M. Patt, Laboratory of Radiobiology, University of California, San Francisco Medical Center, San Francisco, California 94122 (1)
Purdue University, Nuclear Engineering Library, Lafayette, Indiana 47907 (1)
Dr. S. M. Reichard, Director, Division of Radiobiology, Medical College of Georgia, Augusta, Georgia 30902 (1)
University of Rochester, Atomic Energy Project Library, P. O. Box 287, Station 3, Rochester, New York 14620 (1)

OTHER (continued)

Dr. H. H. Rossi, 630 West 168th Street, New York, New York 10032 (1)
Dr. Eugene L. Saenger, Director, Radioisotope Laboratory, Cincinnati General Hospital, Cincinnati, Ohio 45229 (1)
Sandia Corporation Library, P. O. Box 5800, Albuquerque, New Mexico 87115 (1)
Scientific Committee on the Effects of Atomic Radiation, ATTN: Library, United Nations Room 3267, United Nations Plaza, New York, New York 10017 (1)
Scope Publications, Franklin Station, P. O. Box 7407, Washington, D. C. 20004 (1)
Dr. Arthur R. Tamplin, Biophysicist, Information Integration Group, University of California, Lawrence Radiation Laboratory, L-612, Livermore, California 94550 (1)
Radiation Biology Laboratory, Texas Engineering Experiment Station, Texas A. & M. University, College Station, Texas 77840 (2)
Texas Nuclear Corporation, ATTN: Director of Research, Box 9267 Allandale Station, Austin, Texas 78756 (1)
Western Reserve University, Department of Radiology, Division of Radiation Biology, Cleveland, Ohio 44106 (1)
Mr. Lionel Zamore, 601 Brightwater Court, Brooklyn, New York 11235 (1)

FOREIGN

International Atomic Energy Agency, Kaerntnerring 11, Vienna I. 1010, Austria (1)
European Atomic Energy Community, C.E.E.A., Library, 51 rue Belliard, Brussels 4, Belgium (1)
Dr. L. G. Lajtha, Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester, England (1)
Dr. L. F. Lamerton, Biophysics Department, Institute of Cancer Research, Surrey Branch, Belmont, Sutton, Surrey, England (1)
National Lending Library for Science and Technology, Boston Spa, Yorkshire, England (1)
Directorate of Medical and Health Services, FAF (Federal Armed Forces), Bonn, Ermekeilstr. 27, West Germany (1)
Abteilung fur Strahlenbiologie im Institut fur Biophysik der Universitat Bonn, 53 Bonn-Venusberg, Annaberger Weg 15, Federal Republic of Germany (2)
Prof. Dr. H. Langendorff, Direktor des Radiologischen Instituts der Universitat, 78 Freiburg im Breisgau, Albertstrasse 23, Germany (1)
Dr. Helmut Mitschrich, Academie des Sanitaets-und Gesundheits, Weseus BW, Spezialstab ATV, 8 Muenchen Schwere-Reiterstr. 4, Germany (2)
Prof. Dr. F. Wachsmann, Gesellschaft fur Strahlenforschung m.b.H., 8042 Neuherberg bei Muenchen, Institut fur Strahlenschutz, Ingolstadter Landstrasse 1, Muenchen, Germany (1)
Joachim Emde, Col. Director ATV/Stab, ABC- und Selbstschutzschule, SpezStATV/R, 8972 Sonthofen 2/Allgaeu, Berghoferstrasse 17, West Germany (1)
Dr. M. Feldman, Section of Cell Biology, The Weizmann Institute of Science, Rehovoth, Israel (1)
Dr. G. W. Barendsen, Radiobiological Institute TNO, Rijswijk, Netherlands (1)
Puerto Rico Nuclear Center, ATTN: Reading Room, College Station, Mayaguez, Puerto Rico 00708 (2)
Dr. H. Cottier, Pathological Institut der Universitat, Bern, Switzerland (1)

UNCLASSIFIED
Security Classification

**DOCUMENT CONTROL DATA - R&D**

*(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)*

1. ORIGINATING ACTIVITY *(Corporate author)*
Armed Forces Radiobiology Research Institute
Defense Atomic Support Agency
Bethesda, Maryland 20014

2a. REPORT SECURITY CLASSIFICATION
UNCLASSIFIED

2b GROUP
N/A

3. REPORT TITLE
RADIATION-INDUCED ULTRASTRUCTURAL CHANGES IN LYSOSOMES
I. CYTOCHEMICAL ANALYSIS

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*

5. AUTHOR(S) *(Last name, first name, initial)*
René, A. A., Parker, J. L. and Darden, J. H.

| 6. REPORT DATE | 7a. TOTAL NO. OF PAGES | 7b. NO. OF REFS |
|---|---|---|
| December 1969 | 25 | 24 |

| 8a. CONTRACT OR GRANT NO. | 9a. ORIGINATOR'S REPORT NUMBER(S) |
|---|---|
| b. PROJECT NO. | AFRRI SR69-26 |
| c. MC 3 90203 | 9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)* |
| d. | |

10. AVAILABILITY/LIMITATION NOTICES
Distribution of this document is unlimited

| 11. SUPPLEMENTARY NOTES | 12. SPONSORING MILITARY ACTIVITY |
|---|---|
| | Defense Atomic Support Agency<br>Washington, D. C. 20305 |

13. ABSTRACT

Ultrastructural and biochemical changes in lysosomes of rat liver following exposure to ionizing radiation were studied. A marker for acid phosphatase was used to visually correlate the progressive changes in lysosomes with the cellular necrobiotic process postirradiation. The earliest observable change in the lysosomes and/or lysosomal enzymes corresponding with the sequence of fine structural alterations following irradiation suggests that radiation labilizes the lysosomal membrane resulting in a release of enzymes responsible for cell damage. The concentration of the lead phosphate reaction product indicated that the initial action on the lysosome is evidently a "build-up" of hydrolytic enzymes within 2 hours after irradiation followed by a gradual release of the marked enzyme 2-24 hours postirradiation as noted by decreased enzyme concentration within the lysosomes. The release of the enzyme appeared to be directly related to an increasing cellular necrobiosis following irradiation.

DD FORM 1 JAN 64 1473

UNCLASSIFIED
Security Classification

| 14. KEY WORDS | LINK A | | LINK B | | LINK C | |
|---|---|---|---|---|---|---|
| | ROLE | WT | ROLE | WT | ROLE | WT |
| | | | | | | |

INSTRUCTIONS

1. ORIGINATING ACTIVITY: Enter the name and address of the contractor, subcontractor, grantee, Department of Defense activity or other organization *(corporate author)* issuing the report.

2a. REPORT SECURITY CLASSIFICATION: Enter the overall security classification of the report. Indicate whether "Restricted Data" is included. Marking is to be in accordance with appropriate security regulations.

2b. GROUP: Automatic downgrading is specified in DoD Directive 5200.10 and Armed Forces Industrial Manual. Enter the group number. Also, when applicable, show that optional markings have been used for Group 3 and Group 4 as authorized.

3. REPORT TITLE: Enter the complete report title in all capital letters. Titles in all cases should be unclassified. If a meaningful title cannot be selected without classification, show title classification in all capitals in parenthesis immediately following the title.

4. DESCRIPTIVE NOTES: If appropriate, enter the type of report, e.g., interim, progress, summary, annual, or final. Give the inclusive dates when a specific reporting period is covered.

5. AUTHOR(S): Enter the name(s) of author(s) as shown on or in the report. Enter last name, first name, middle initial. If military, show rank and branch of service. The name of the principal author is an absolute minimum requirement.

6. REPORT DATE: Enter the date of the report as day, month, year; or month, year. If more than one date appears on the report, use date of publication.

7a. TOTAL NUMBER OF PAGES: The total page count should follow normal pagination procedures, i.e., enter the number of pages containing information.

7b. NUMBER OF REFERENCES: Enter the total number of references cited in the report.

8a. CONTRACT OR GRANT NUMBER: If appropriate, enter the applicable number of the contract or grant under which the report was written.

8b, 8c, & 8d. PROJECT NUMBER: Enter the appropriate military department identification, such as project number, subproject number, system numbers, task number, etc.

9a. ORIGINATOR'S REPORT NUMBER(S): Enter the official report number by which the document will be identified and controlled by the originating activity. This number must be unique to this report.

9b. OTHER REPORT NUMBER(S): If the report has been assigned any other report numbers *(either by the originator or by the sponsor)*, also enter this number(s).

10. AVAILABILITY/LIMITATION NOTICES: Enter any limitations on further dissemination of the report, other than those imposed by security classification, using standard statements such as:

(1) "Qualified requesters may obtain copies of this report from DDC."

(2) "Foreign announcement and dissemination of this report by DDC is not authorized."

(3) "U. S. Government agencies may obtain copies of this report directly from DDC. Other qualified DDC users shall request through _____."

(4) "U. S. military agencies may obtain copies of this report directly from DDC. Other qualified users shall request through _____."

(5) "All distribution of this report is controlled. Qualified DDC users shall request through _____."

If the report has been furnished to the Office of Technical Services, Department of Commerce, for sale to the public, indicate this fact and enter the price, if known.

11. SUPPLEMENTARY NOTES: Use for additional explanatory notes.

12. SPONSORING MILITARY ACTIVITY: Enter the name of the departmental project office or laboratory sponsoring *(paying for)* the research and development. Include address.

13. ABSTRACT: Enter an abstract giving a brief and factual summary of the document indicative of the report, even though it may also appear elsewhere in the body of the technical report. If additional space is required, a continuation sheet shall be attached.

It is highly desirable that the abstract of classified reports be unclassified. Each paragraph of the abstract shall end with an indication of the military security classification of the information in the paragraph, represented as *(TS)*, *(S)*, *(C)*, or *(U)*.

There is no limitation on the length of the abstract. However, the suggested length is from 150 to 225 words.

14. KEY WORDS: Key words are technically meaningful terms or short phrases that characterize a report and may be used as index entries for cataloging the report. Key words must be selected so that no security classification is required. Identifiers, such as equipment model designation, trade name, military project code name, geographic location, may be used as key words but will be followed by an indication of technical context. The assignment of links, rules, and weights is optional.

GPO 885-447